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Microbial contamination assessment and identification of enterobacteriaceae isolated from the surface water of Govantes River, Vigan City, Ilocos Sur, Philippines

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Abstract

The world is currently grappling with significant challenges concerning the availability and quality of freshwater resources, which profoundly impact human life. Water-borne diseases remain a leading cause of morbidity in the Philippines. This study assessed the microbiological contamination of the Govantes River in Vigan City, which serves multiple uses, including domestic and agricultural consumption, primary production, transportation, and recreation. Multiple-tube fermentation technique was employed to determine the coliform prevalence, and the Vitek 2 system was used to identify isolated Enterobacteriaceae. The bacteriological analysis revealed that the river is highly contaminated with total and fecal coliforms during the dry and wet seasons. *Klebsiella pneumonia* (38.10%) was the most prevalent isolate, followed by *Providencia stuartii* (28.57%), AmpC β-lactamase-producing *Enterobacter cloacae* (23.81%), and AmpC β-lactamase-producing *Klebsiella pneumoniae* (9.52%). The consistently high coliform loads across all sampling stations indicate severe contamination, and the presence of AmpC β-lactamase-producing bacteria is particularly alarming. These findings emphasize the need for comprehensive strategies to revitalize and restore the river system to protect public health.

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Introduction

The presence of waterborne pathogens in rivers is one of the significant threats to public health with a high economic burden (Pandey *et al.*, 2014; Ramírez-Castillo *et al.*, 2015; Nhantumbo *et al.*, 2023. Waterborne diseases cause substantial morbidity and mortality globally, primarily affecting the very young in the poorest countries (Hunter, 2012). In the Philippines, the leading waterborne diseases are acute bloody diarrhea, cholera, rotavirus, hepatitis A, and typhoid (Corpuz, 2021). In addition, water consumption from unsafe and uncertain sources has indicated different health risks such as cancer, nephrotoxicity, central nervous system effects, and even cardiovascular diseases (Su, 2006). The prevalence of waterborne diseases in developing countries is attributed to the lack of safe water access, poor hygiene, and environmental factors (Forstinus *et al.*, 2016).

Surface waters, including rivers and streams, represent an essential water source for drinking, household, agriculture, recreational activities, and other uses. However, they are vulnerable to pollution and frequently contaminated with feces (Kolarevic *et al.*, 2011). The Govantes River, located in Vigan City, Ilocos Sur, Philippines, is an integral part of the region's rich cultural and historical landscape (Tabunan, 2019; Rojas, 2023). Despite being suitable for fishery and agriculture, Vigan City rivers are facing declining water quality due to rapid urban development. This is a common issue in many metropolitan areas, where the city's growth leads to increased pollution and degradation of water bodies (Rini *et al.*, 2020). Despite the increasing body of research on freshwater ecosystems, studies have yet to evaluate the microbial contamination of the Govantes River in Vigan City.

Human impact, human-related activities, and population explosion have dramatically affected the aquatic environment (Páll *et al.*, 2013). Industrial and domestic waste disposal, have significantly impacted the health of rivers and other inland water ecosystems (Mishra and Tripathi, 2007). The presence of fecal coliform in water sources is a crucial indicator of recent fecal contamination and a potential risk to urban populations (Widmer *et al.*, 2013). Studies have consistently found the presence of Enterobacteriaceae, a family of bacteria commonly associated with the gastrointestinal tract of humans and animals, in urban rivers (Lihan *et al.*, 2017). These bacteria indicate fecal contamination and can pose significant health risks, particularly in areas with high human population densities (Paulse *et al.*, 2012). Identifying various Enterobacteriaceae species in these rivers, some resistant to antibiotics, further emphasizes the potential public health implications (Lihan *et al.*, 2017). This has led to various problems, including water pollution, loss of biodiversity, changes in species behavior, eutrophication, and human health risks (Bougherira *et al.*, 2014); (Ullah Bhat and Qayoom, 2022). The Philippines, in particular, has been affected by the absence of an effective system to control the dumping of untreated sewage, garbage, and industrial effluents into water bodies (Pleto *et al.*, 2020). The difficulties in implementing environmental laws further intensify the situation (Migo *et al.*, 2018).

Rivers are essential freshwater resources for domestic and industrial activities, and the availability of good-quality freshwater is indispensable for preventing water-borne diseases and improving quality of life, especially in communities that lack a water supply system (Titilawo *et al.*, 2019). It plays an essential role in human consumption and development and is vital to a city's economic and sustainable development. Hence, this study is conducted to assess the microbial contamination of the Govantes River and identify the Enterobacteriaceae present. This provides critical insights into the river's state of microbial contamination and the presence of waterborne pathogens. The result of the study shall serve as a basis for crafting strategies to revive, rehabilitate, and protect the river system. In addition, the study shall also serve as a basis for how and for what water can be used and the species and ecosystem it can support. Lastly, it also provides information on identifying actual and emerging diseases, problems of water pollution, and land use, formulating plans, and evaluating the effectiveness of the different programs and projects to rehabilitate these rivers.

Materials and methods

Study site

Sampling sites were selected based on surrounding activities, such as agriculture, recreation, and population density, ensuring a comprehensive representation of the river's different sections. Samples were collected from upstream, midstream, and downstream areas, specifically at Brgy. Amianance (upstream), Brgy. Pantay Daya (midstream), and Brgy. Pantay Laud (downstream) (Fig. 1). Considerations for site selection included tributary characteristics, runoff, and point and non-point sources of pollution (Hou *et al.*, 2022).

Fig. 1. Govantes River showing the sampling stations (Site 1: Brgy. Amianance, Site 2: Brgy. Pantay Daya and Site 3: Pantay Laud.

Sample collection

Clear, wide-mouth glass bottles with heat-resistant plastic screw caps, each holding 200 mL, were used for sample collection. Before collection, bottles were cleaned with detergent and tap water, rinsed with distilled water, covered with aluminum foil, and sterilized by autoclaving at 121°C.

Surface water samples were collected during the wet season (January) and rainy season (June) of 2022. Composite sampling involved collecting one sample from the middle of the river and two from the coastal zones on opposite sides mixed. Samples were collected by submerging the bottles downstream to 30-50 cm depth, filling them with 150 mL of water, and leaving a 2.5 to 5 cm headspace. The samples were then stored in an icebox and promptly transported to the Microbiology and Biotechnology Laboratory at the University of Northern Philippines.

Bacteriological analysis

Surface water samples were analyzed using the Multiple Tube Fermentation (MTFT) technique to detect total and fecal coliforms, adhering to the APHA and DENR-EMB standards. The presumptive test involved inoculating Durham fermentation tubes with lactose broth and water samples, followed by incubation at 36°C for 24-48 hours. Positive tests indicated by gas formation were subjected to confirmatory testing using brilliant green lactose bile

(BGLB) broth and Escherichia coli (EC) broth. Confirmed positive samples were used to calculate coliforms' most probable number (MPN) (Grasso *et al.*, 2000).

All positive confirmatory tests were sub-cultured on Eosin Methylene Blue (EMB) agar and incubated at 36°C for 24 hours (Geletu *et al.*, 2022). Pure cultures were identified using the Vitek 2 system at Mariano Marcos Memorial Hospital and Medical Center.

Results and discussion

Bacteriological analysis of surface water and identification of isolated Enterobacteriaceae collected from the Govantes River, Vigan City, were conducted to evaluate microbial contamination and the potential health risks of the urban river.

Prevalence of total and fecal coliform

The bacteriological analysis of the surface waters from the three collection sites along the Govantes River indicates significant contamination with high total and fecal coliform bacteria (Fig. 2). The upstream section of the Govantes River at Brgy. Amianance exhibited the highest levels of total coliform and fecal coliform bacteria, exceeding 1600 MPN/100ml during the dry and wet seasons. The lowest contamination during the dry season was observed downstream at Brgy. Pantay Laud, with total coliform levels at 79 MPN/100ml and fecal coliform levels at 49 MPN/100ml. During the wet season, the midstream section at Pantay Daya recorded the lowest contamination levels, with both total and fecal coliform bacteria measured at 20 MPN/100ml.

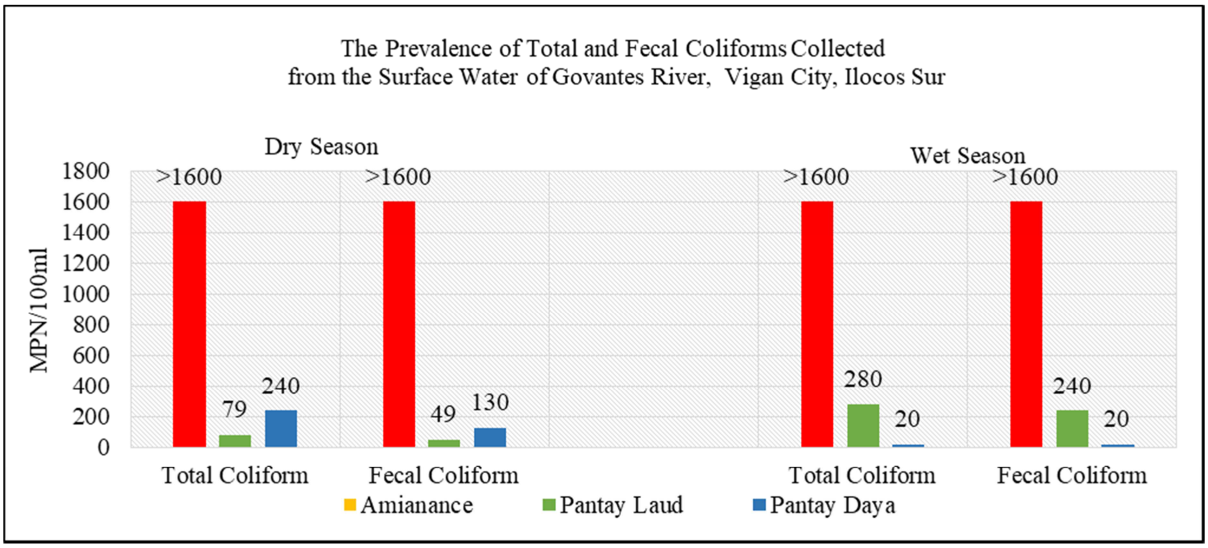

Fig. 2. The prevalence of total and fecal coliform collected from the surface water of Govantes River, Vigan City

These results indicate substantial microbial contamination throughout the Govantes River, with notable variations in contamination levels depending on the location and season. The high levels of coliform bacteria, especially in the upstream areas, suggest significant sources of pollution, potentially from residential, agricultural, or industrial activities. The presence of fecal coliforms indicates contamination from fecal matter, which poses health risks to humans and animals utilizing these water resources. These findings underscore the need for interventions to improve water quality and mitigate pollution sources along the Govantes River.

The high prevalence of total and fecal coliforms in the upstream section of the Govantes River is a significant finding supported by previous research. Fecal coliform and fecal streptococci levels are often higher at upstream sites influenced by discharges from combined sewer outfalls (Murray *et al.*, 2001). This aligns with the conditions observed in the Govantes River, the upstream area. Numerous establishments, including government offices, corporate facilities, academic institutions, tourist attractions, and residences, surround Brgy. Amianance. Several factors contribute to the high levels of microbial contamination upstream. The increasing population and influx of tourists in hotels and restaurants along the riverbanks contribute significantly to microbial contamination. The presence of many establishments worsens the discharge of untreated sewage and domestic waste into the river.

Many areas need proper sanitation infrastructure, resulting in the direct discharge of untreated wastewater into the river. Domestic waste from various establishments, residential houses, and industrial effluents is often funneled into open drains and gutters, eventually leading to the river. This pattern of contamination is not unique to the Govantes River. Similar issues in the Buhisan, Bulacao, and Lahug Rivers in Cebu were found where organic pollution and coliform presence were linked to high-impact human activities (Bensig *et al.*, 2014). Similarly, it was observed that highly urbanized areas in Suzhou, China had significantly higher levels of thermotolerant coliforms due to rapid urbanization (Yuan *et al.*, 2019). In addition, the study of the Diyala River highlighted significant water quality issues, particularly due to urban influences from the Rustumiya sewage treatment plant, which contributed to the river's deterioration (Hadi, 2023). Similarly, rapid urbanization in Cameron Highlands is expected to exacerbate problems related to water and other resources (Weng, 2017).

The downstream section of the Govantes River along Brgy. Pantay Laud showed the lowest levels of total and fecal coliforms during the dry season. This can be attributed to the fact that human settlements are further from the downstream area, which is primarily used for aquaculture. Numerous fish pens indicate less human activity and waste discharge in this section. Additionally, the river flow is not continuous from upstream to downstream during the dry season, causing pollutants to accumulate upstream and reducing contamination downstream. During the wet season, the midstream section at Pantay Daya recorded the lowest prevalence of total and fecal coliforms. This is likely due to the increased river flow during the wet season, which helps to transport microbial loads and contaminants from the upstream areas downstream. As a result, contaminants are more dispersed, leading to lower concentrations in the midstream area.

The high population density, intensive industrial activity, and extensive agricultural practices in Vigan City contribute significantly to anthropogenic pollution in the Govantes River. The increasing abundance of pollution indicator bacteria, such as total and fecal coliforms in river water, is widespread in urban and rural regions. This often leads to outbreaks of waterborne diseases such as cholera and dysentery. Addressing these pollution sources is critical for improving water quality and safeguarding public health.

Enterobacteriaceae present in the surface water of Govantes river

The study highlights the significant accumulation of waterborne diarrheal bacteria in the Govantes River, which poses a severe risk of contaminating drinking and irrigation water. In urban areas, water bodies and soils are frequently exposed to substantial discharges of contaminants, including fecal bacteria of both human and non-human origin. These bacteria often carry genes for antibiotic resistance, complicating public health efforts (Guzman-Otazo *et al.*, 2019). The genera *Citrobacter, Enterobacter, Escherichia, Klebsiella, Morganella, Plesiomonas, Proteus, Providencia, Salmonella, Serratia, Shigella*, and *Yersinia* include species that are clinically significant and frequently cause infections. These infections range from pneumonia, enteritis, diarrhea, and septicemia to wound infections and central nervous system infections (Janda and Abbott, 2021).

Enterobacteriaceae are typically present in the intestines of humans and animals and are the most reliable sign of fecal contamination in water, indicating the potential presence of pathogens.

As shown in Fig. 3, four Enterobacteriaceae species were isolated and identified from the water samples: *Klebsiella pneumoniae*, *Providencia stuartii*, AmpC β-Lactamase producing *Enterobacter cloacae*, and AmpC β-Lactamase producing *Klebsiella pneumoniae*.

K. pneumoniae was the most prevalent species, accounting for 38.10% of the isolates. This bacterium is known for causing various infections, including pneumonia, urinary tract infections, and bloodstream infections (Corpuz, 2020). *P. stuartii* followed with 28.57% prevalence. This species is often associated with urinary tract infections, particularly in catheterized patients. AmpC β-Lactamase producing *E. cloacae* represented 23.81% of the isolates. These bacteria are significant due to their resistance to many β-lactam antibiotics, complicating treatment options. AmpC β-Lactamase producing *K. pneumoniae* accounted for 9.52%. The presence of β-lactamase producing strains highlights the potential for antibiotic-resistant infections. *P. stuartii* was most prevalent in Brgy. Amianance and Pantay Laud, suggests different contamination sources or environmental conditions favoring this bacterium in these areas. *K. pneumoniae* and AmpC Beta-Lactamase-producing strains were most prevalent in Pantay Daya and Pantay Laud, indicating these areas might have conditions conducive to the survival and proliferation of these more resistant and pathogenic bacteria.

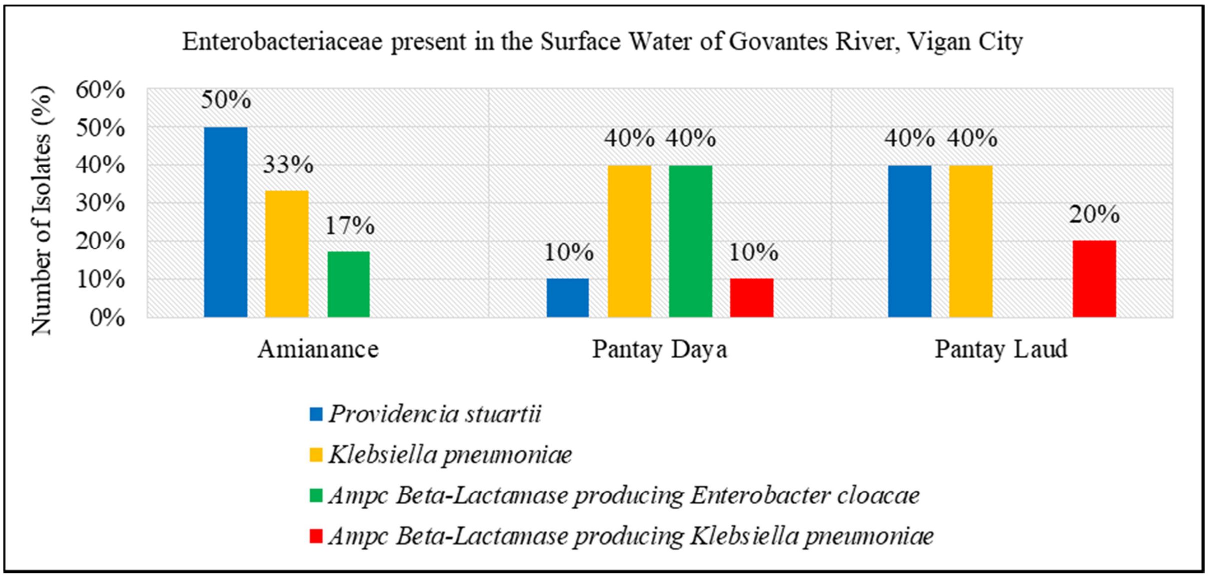

Fig. 3. Enterobacteriaceae isolated from the surface water of Govantes River, Vigan City

The study noted a high prevalence of *K. pneumoniae* and AmpC β-Lactamase-producing *K. pneumoniae* in the surface water of the Govantes River. *K. pneumoniae* is an opportunistic pathogen known for causing various hospital-acquired infections, including pneumonia, septicemia, and urinary tract infections. These infections contribute significantly to morbidity and mortality, especially among patients with chronic illnesses. The pathogenicity of *K. pneumoniae* is enhanced by various virulence factors and genetic determinants of antibiotic resistance, making treatment challenging and posing a significant threat to the healthcare system (Ssekatawa *et al.*, 2021). A similar study by Podschun *et al.* (2001) found that 53% of surface water samples tested positive for *Klebsiella* species, with *K. pneumoniae* being the most common. Their data showed that surface water isolates of *K. pneumoniae* expressed virulence factors similar to clinical strains. *P. stuartii* was also prevalent in the water samples. *P. stuartii* is a notable cause of catheter-associated urinary tract infections, especially in elderly patients with long-term indwelling urinary catheters. Though *Providencia* species are not frequent causes of urinary tract infections or bacteremia, such infections can result in high mortality rates, particularly in elderly patients with severe underlying conditions. *P. rettgeri* and *P. stuartii* are commonly found in water, soil, and animal reservoirs, acting as opportunistic pathogens in hospitalized patients and elderly residents in nursing care facilities (Wie, 2015). Enterobacter species such as *E. aerogenes* and *E. cloacae* have emerged as important opportunistic and multi-resistant pathogens over the past three decades, particularly in hospitals. These Gram-negative bacteria have been implicated in numerous outbreaks of hospital-acquired infections in Europe, notably France.

Their dissemination is facilitated by regulatory mechanisms that control membrane permeability and express detoxifying enzymes involved in antibiotic resistance. Additionally, these bacteria can acquire genetic mobile elements that enhance their resistance. Their adaptability allows them to colonize various environments and hosts, adjusting their metabolism and physiology to external conditions and stresses (Davin-Regli and Pagès, 2015).

Conclusion

This research revealed a high prevalence of total and fecal coliforms, including pathogenic and antibiotic-resistant bacteria, in the Govantes River in Vigan City. This significant contamination poses a grave threat to public health, emphasizing the need for comprehensive and immediate attention to the intersection of water, food, and health safety.

Recommendation(s)

The findings underscore the urgent need for comprehensive plans to revitalize, repair, and safeguard the river system serving Vigan City. Effective waste management from hospitals, municipalities, agricultural activities, and industries is crucial. Proper treatment and disposal of waste, especially that harboring antibiotic-resistant bacteria, are vital to controlling pathogens' spread.

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